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ABBREVIATIONS

3Rs: Replacement, reduction and refinement of tests that use animals.

BMD: A dose or concentration that produces a predetermined change (e.g. 10%) in the response rate of an adverse effect.

CONCAWE: The organisation of environmental science for the European refining industry.

Create2Solve: An initiative involving a call for viable, animal-free research methods to meet challenges and demands for innovation coming from the world of business.

CRACK IT: Accelerates the availability of technologies which will deliver: i) measurable 3Rs impacts; ii) new marketable products; and iii) more efficient business processes.

ECETOC: European Centre for Ecotoxicology and Toxicology of Chemicals.

GTL: Gas-to-liquid substances produced by Fischer-Tropsch synthesis.

HESI: Health and Environmental Sciences Institute.

LC50: The lethal concentration required to kill 50% of the population.

NC3Rs: UK National Centre for the replacement, refinement and reduction of animals in research, which is behind CRACK IT.

PAH: Polycyclic aromatic hydrocarbons.

POD: Point of departure: the point on a toxicological dose-response curve established from experimental data or observational data that generally corresponds to an estimated low-effect level or no-effect level.

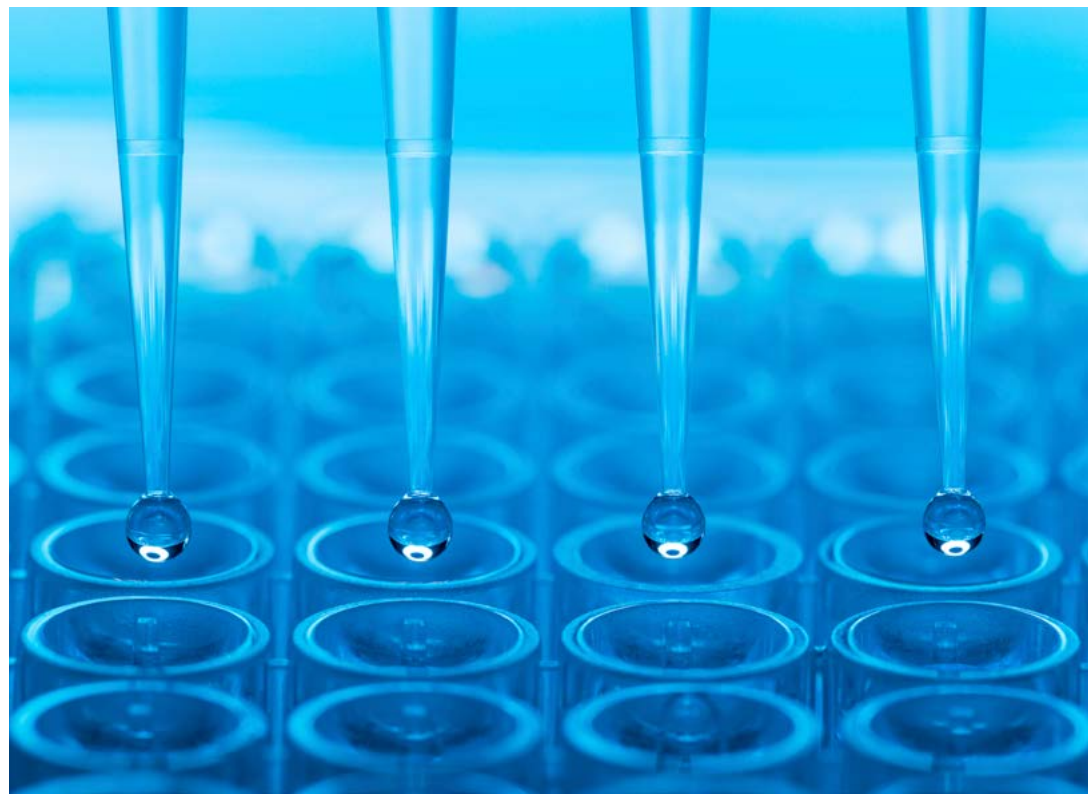
QSAR: Quantitative structure-activity relationship model.

REACH: European Union regulation No. 1907/2006 concerning the registration evaluation, authorisation and restriction of chemicals.

RfD (RfC): RfD is an established daily oral or dermal dose derived from a toxicological data set and conservatively adapted to a human population (including sensitive subgroups) indicating an exposure level that is considered to be free of any appreciable risk of deleterious effects during a lifetime. Its unit is usually mg/kg bw/day. When inhaled, it is called a reference concentration, RfC, usually given as mg/m³.

TTC: Threshold of Toxicological Concern. The TTC is a science-based pragmatic tool for prioritizing those chemicals with low-level exposures that require more data over those that can be presumed to present no appreciable human health risk.

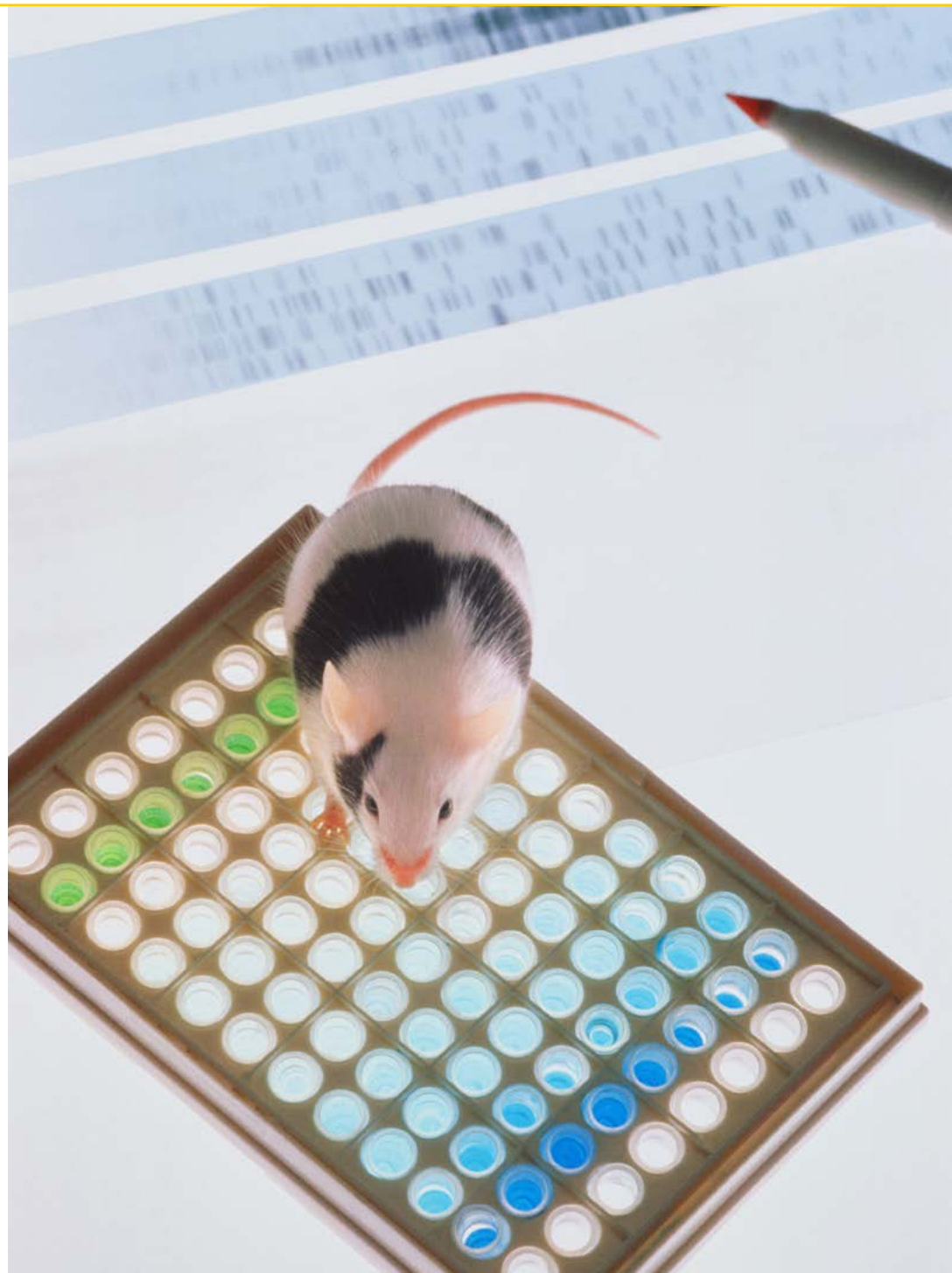
UVCB: Substances of unknown or variable composition, complex reaction products and biological materials.



CHARLES GENTRY

It was with great sadness that Shell learned of the death of Charles Gentry on August 26, 2018. Charles chaired the Shell Animal Welfare Panel from 2010 to 2018, contributing enormously to the development of Shell's policy on animal welfare. His contributions to the Panel, and to animal welfare in general, are highlighted in this Report. He will be greatly missed.

Charles Gentry Award, [page 6](#)



EXECUTIVE SUMMARY

Shell¹ continues to work to end the need for animal testing, using a strategy based on the 3Rs principles: replacement, reduction and refinement. Shell must ensure that any alternative safety evaluation enables it to comply with regulatory requirements and continue to innovate, develop and maintain safe new products and technologies.

The data presented in this Animal Welfare Report 2019 (the "Report") are for vertebrate animal use by Shell worldwide. The main reason for Shell's use of animals remains regulatory compliance, especially with respect to effluent testing in the USA and Canada, and chemical safety testing for the European Union (EU) chemical safety regulation (REACH). Where possible, regulatory compliance tests are conducted with other companies that also seek to comply with the same regulations. This avoids unnecessary duplication of animal tests and minimises the overall use of animals.

Effluent testing is the largest driver of animal use for regulatory purposes. A priority, therefore, is to explore new approaches that can use non-vertebrate testing to identify ecotoxic hazards of chemicals and effluents. In 2019, we have continued our efforts to tackle this issue, including a multi-year analysis of all of our fish effluent data in order to identify opportunities for improvement.

Another priority is to optimise the methods for applying products in *in vitro* tests. Many petroleum derived substances are difficult to test because they are complex multi-constituent substances and/or substances with low water solubility. Current research is directed towards exploring different dosing for complex substances that can enable increased throughput of non-vertebrate toxicity tests.

Other work has centred on further developing the use of biological indicators which reflect the mode of action, traditional physico-chemical properties and manufacturing process description of complex petroleum substances. If successful, this will allow a more accurate grouping of substances on the basis of biology and chemistry, and significantly reduce the use of animals. The approach was refined and applied to the full set of petroleum substances registered under REACH (www.concawe.eu/cat-app). This improved tool for assessing petroleum substances is being actively discussed with regulators in the hope of giving them an alternative to single-substance hazard assessments that use thousands of animals.

Shell actively collaborates with universities and industry consortia to develop knowledge and tools that help reduce animal testing. By presenting Shell's research at conferences and in peer-reviewed journals, Shell is helping to increase the demand for global regulatory acceptance of such alternative methods. Where required by law, Shell has evaluated product safety using animals, but has sought where possible to ensure that the test outcomes have been used to validate alternative methods that avoid animal testing.

¹ Royal Dutch Shell plc is a legal entity and the ultimate shareholder in all Shell companies. The Shell Group, Shell or simply the Group are used interchangeably in this document where references are made to Royal Dutch Shell plc and the companies in which it holds a direct or indirect controlling interest and in general and where no useful purpose is served by identifying the particular company or companies.

CHARLES GENTRY AWARD

This award is given to any individual, team, or corporate entity that has contributed to Shell's commitment to eliminate the need for animal testing and in the meantime to improve the welfare of those animals that are used. The Charles Gentry award aims to encourage the replacement of animal testing by alternatives that will allow the continuing use, innovation and development of safe products and technologies.

The award recognises a significant contribution towards furthering this aim by applying the 3Rs ([see page 9](#)) to Shell's use of animals, in order to:

- replace animal tests with alternative methods whenever possible;
- reduce the number of animals used in any animal tests for which there are no alternative methods; and
- refine animal test methods to make them as humane as possible.

The award is given in memory of Charles Gentry who served from 2010 to 2018 as chair of Shell's Animal Welfare Panel, a panel of independent experts which reviews and reports on Shell's animal testing activities. In this role, he contributed enormously to the development of Shell's policy on animal welfare, particularly towards ensuring that contractors maintained high standards of animal care while researching or testing on Shell's behalf.

Charles had extensive experience in laboratory animal science. He was dedicated to improving the welfare of animals used in research, and to encouraging the development and education of animal



technicians at institutions where he worked. He actively encouraged the uptake of refinements in animal care and use, helping to spread good practice throughout the sector.

Charles was a first-rate chairman who is remembered for his achievements, his knowledge and his exceptional personality, which he used to achieve change for the better by consensus.

He will be greatly missed.

The 2019 Charles Gentry Award has been given to: Graham Whale. Throughout a career that has included 29 years at Shell, Graham has been a tireless advocate for alternative testing methods to evaluate hazards of petrochemical substances and industrial effluents. He has conducted and supervised work within Shell and industry organisations that showed the effectiveness of alternative approaches, especially read-across and weight-of-evidence techniques. These methods have been applied with great effect for chemical registration under EU REACH and similar product regulatory frameworks in other regions. Graham has also chaired industry groups where he has been instrumental in developing whole effluent risk-based assessment (RBA) methods that avoided the use of vertebrates (fish) while examining the risk of effluent discharges. These methods have been adapted for assessing offshore discharges with minimal fish testing, becoming part of the Shell “toolbox” for tiered effluent risk assessment. Graham has also made significant contributions to other research and development programmes that focused on alternatives to animal testing. He has been active in such groups as the UK NC3Rs Ecotoxicity Working Group and the HESI (Health and Environmental Sciences Institute) Animal Alternatives in Environmental Risk Assessment Committee, in which he has played an important role in engaging with regulators and making the case for non-animal alternatives.

During his time at Shell, he was pre-eminent within the company in advocating for alternatives to animal testing and championing efforts in this area. Graham, who is now working as an independent



consultant, has authored more than 150 external publications and Shell technical reports on the environmental risk assessment of chemicals and effluents. He has made presentations within the Shell Group, to regulatory authorities, international conferences for the oil and gas industry, expert working groups, scientific conferences and Royal Societies.

It is therefore a great pleasure for Shell to present the 2019 award to Graham.

INTRODUCTION

There are strong ethical, scientific and business reasons to move away from animal testing as the primary methodology for demonstrating safety. For the time being, however, we live in a strictly regulated environment where animal testing is still required to support the safety of Shell's processes and products.

The 3Rs (replacement, reduction and refinement) are now broadly accepted as the fundamental ethical framework within which animal research should be conducted. Replacement means using insentient material instead of conscious living higher animals. Reduction means lowering the number of animals used to obtain information of the required amount and precision; Refinement refers to any measure that decreases the severity of procedures applied to those animals which still have to be used, (and includes the provision of better housing and husbandry).

Shell applies the 3Rs principles in its product safety assessments wherever possible, while meeting legal obligations and protecting human life and the environment. Any Shell-owned or Shell-operated company must follow the company's animal testing standards when performing laboratory-based ecotoxicology studies on animals, even in countries with less stringent requirements. Under Shell's standards, animal testing remains the last resort. Using non-animal tests to generate equivalent information is always the first choice.

Replacement Reduction Refinement

At least twice a year, the external Animal Welfare Panel ("the Panel"), examines and comments on the implementation of Shell's animal testing requirements. The Panel works with Shell to ensure good practice in laboratories. It also advises on how Shell should optimise its engagement externally with the development and application of the 3Rs throughout the company's activities. Membership and terms of reference of the Panel are provided at the end of this Report.

This Report details Shell's ongoing efforts to replace, reduce and refine animal testing by advancing new and alternative testing methods (e.g. in vitro tests). It describes Shell's external engagement and advocacy for alternatives to traditional animal experimental methods. The Report also contains an overview of Shell's use of animal testing to assess the safety characteristics and environmental impact of its products, operations and manufacturing processes.

This Report has been reviewed and approved by the Panel.

3RS ACTIVITIES FOR ANIMAL WELFARE

The landscape of animal testing in 2019

The main driver for animal use in Shell is the need to comply with regulations and the law when determining the hazard that chemicals pose to humans and the environment.

Regulatory compliance requires testing on animals to satisfy chemical hazard assessment, regardless of the risk of exposure to a substance. This focus on the intrinsic hazard of chemicals means that animal testing guidelines are frequently updated and extended to cover potential new hazards. This results in more animals being needed for the new tests. The updates also mean that chemicals may need to be re-tested because previous studies might be deemed “inadequate” on the grounds that they were performed under “outdated” protocols. While Shell sees chemical safety evaluation as paramount, we strongly believe that ever-increasing animal testing does not necessarily improve our overall safety assessments. Existing scientific knowledge and alternative methods can be combined to assess potential new hazards and minimise animal use.

Regulatory frameworks, (e.g. REACH in the EU), depend heavily on animal tests following primarily OECD guidelines. A strict interpretation of the regulatory requirements may result in focusing solely on the animal test itself, rather than the outcome that the regulation aims to achieve by requiring the animal test. A focus on the outcome of chemical safety assessment will create opportunities exploring novel and alternative ways of assessing chemical safety while benefiting animal welfare.



The Shell strategy on animal testing can be described by reference to four main activities that support the principles of the 3Rs (replacement, reduction and refinement). Each activity describes the mindset and behaviours that guide Shell’s subject matter experts (SMEs) on animal welfare as they seek to create and practise a culture of critical improvement and care. They select their priorities according to Shell’s responsibilities to assess human and environmental safety. They also pay attention to overcoming barriers to the progress of the 3Rs for animal tests. The four main areas of activity are:

Research and develop, which refers to efforts at collaborating on, funding and conducting research into innovative ways of assessing hazard and exposure. Drivers for prioritisation are business needs, and areas where the highest impact on the 3Rs can be achieved.

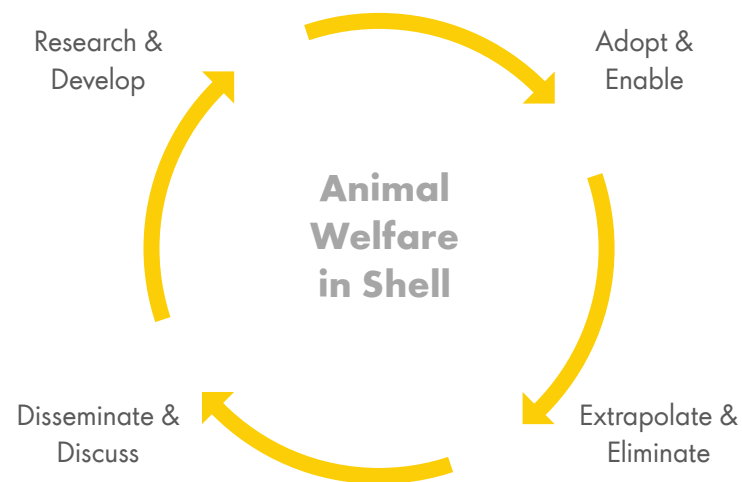
Adopt and enable, which refers to absorbing external good practice, research advances and new knowledge into Shell’s ways of working. Shell implements the advances and insights into its

assessments of hazard and exposure. By promoting a culture of care in industry organisations where Shell is active, we also aim to identify and adopt best practices for animal welfare and to reduce animal testing in product safety and regulatory compliance.

Extrapolate and eliminate, which minimises or eliminates animal testing through collaboration and the use of existing data and prediction models. By establishing, using and maintaining access to databases, it becomes possible to integrate information from multiple sources. Internally gained insights are extrapolated to external applications to build confidence in the innovative methods. For this activity to succeed, collaboration with external parties is essential.

Disseminate and discuss includes publishing results, presenting data and ideas in professional fora, and engaging with regulators and key academics. It also includes the sharing of good practice, and the review of acquired knowledge by peers and an external panel. This approach aims to instil a culture of care at the highest scientific and practical level, and potentially to reduce animal testing. It also seeks to achieve a wide acceptance of insights and to generate new ideas that can be used in practice.

The following sections of this Report highlight Shell's efforts and progress in each of these activities.



RESEARCH AND DEVELOP

3Rs elements: replace

In 2019, Shell in collaboration with two other chemical companies proposed a proof-of-concept project to the Netherlands Organisation for Health Research and Development (ZonMw), which funds health research and promotes the use of the resulting knowledge.

The proposal was submitted under ZonMw's Create2Solve programme, which supports the development of animal-free innovations that lead to commercial methods, models or services. Entitled Better In Vitro Dosing (BID): Framework and technology development for improving the quality of in-vitro data, the proposal sought to develop a framework and new technologies for better dosing and understanding of exposure within in vitro test systems.

Most petroleum-derived substances and many petrochemicals are difficult to test because they are highly complex, comprising numerous different molecules. These complex substances are called UVCBs (Unknown or Variable composition, Complex reaction products and Biological materials).

Developing animal-free testing methodologies for UVCBs remains a focus of our research because there is currently a shortage of available techniques. To date, most novel animal-free testing methodologies rely on water-based test systems, making them unsuitable for UVCBs, which are poorly water-soluble.



Many of Shell's substances fall into the UVCB category, so improvements would significantly assist the uptake of in vitro methods for our substance types.

The project was awarded to a consortium led by Dr Nynke Kramer (Utrecht University, Institute for Risk Assessment Sciences) and began in early 2020. Further details can be found at <https://www.zonmw.nl/nl/actueel/nieuws/detail/item/three-project-groups-take-on-industry-challenges-to-develop-animal-free-innovations>.

3Rs Benefit

A better understanding of in vitro dosing should enable greater and more effective use of in vitro methods for 'difficult-to-test substances', and thus may help replace the use of animals.

Research and Development of new hazard assessment approaches – developmental toxicity

3Rs elements – reduce and replace

The aim of this activity is to reduce the number of animals tested by:

1. grouping similar substances into “families”, commonly referred as “categories”;
2. reading-across existing hazard information from one known data-rich substance to another data-poor but similar substance; and
3. developing innovative non-animal-testing methods to replace in vivo methods.

Prenatal development tests were conceived in the 1960s, initially as a method to screen for chemicals that may affect the development of a foetus. This requires exposing pregnant animals to chemicals through gestation and assessing the impact of the exposures on the offspring after they are born. These tests use a high number of animals and are required by law.

Our research and development strategy focuses on developing a grouping methodology for UVCBs and animal-free testing methodologies that are applicable to UVCB substances.

The purpose is to develop a targeted testing strategy that uses a minimum of animal testing to identify substances with potential prenatal developmental effects (i.e. toxic effects on the developing embryo). We have developed a mechanism-based hypothesis

approach that focuses on specific parts of petroleum products which have distinct effects on the developing foetus.

Most petroleum substances are produced in volumes of more than 1,000 tonnes a year, meaning REACH regulations require investigations into how they may affect the prenatal development of two species, usually rats and rabbits. This would result in more than 3,000 animals being tested per substance, which would multiply by the thousands of individually registered substances under REACH.

Testing each individual substance is clearly impractical and from the animal welfare point of view unacceptable. However, the REACH regulatory framework requires such tests to be performed. This poses a great challenge because many petroleum and petrochemical substances are UVCB.

Our current efforts are directed towards understanding how petroleum substances affect prenatal development. To reduce the number of animal tests needed, petroleum UVCB substances have been grouped into product families (categories) based on composition, refining history, and known commonality in hazards.

Grouping substances into categories for chemical safety assessment is based on the hypothesis that similar substances have similar toxicity or a predictable trend in toxicity. The existing or newly generated hazard information can be read-across from one substance to another of the same category, provided there is sufficient basis to assume that these substances have similar profiles.

3Rs Benefit

Read-across avoids duplication of data and significantly reduces the numbers of animals that would otherwise be required.

Establishing a mechanism of action for targeted hazard assessment

One of the hypotheses currently being investigated for read-across is based on chemical compositional differences between petroleum substances.

The investigated hypothesis is that polycyclic aromatic hydrocarbons (PAHs) which can be present in some petroleum substances may cause prenatal developmental toxicity by interacting with specific genes involved in foetal development. The hypothesis was tested in a series of in vitro cell systems that were exposed to PAH extracts of petroleum substances with different levels of PAH. The results obtained were compared to those for PAH-free synthetic analogue gas to liquid (GTL) products. This showed that the tested PAH extracts of petroleum substances may have biological modulatory activities associated with the quantity and type of PAHs present. By contrast, these modulation activities were absent in all the PAH-free synthetic products tested.

At the cellular level, specific gene activity associated with PAH correlates with in vitro prenatal developmental toxicity effects. This suggests that PAH toxicity has an important modulating effect in foetal development.

When the results of in vitro activities and chemical composition of petroleum products are combined, it is observed that types of petroleum substances may be assigned their own "signature" based on their PAH profile. In this context, a substance's signature is understood as a distinct response that is biologically and chemically

characterised and allows differentiation from other substances. Knowledge of PAH levels in a petroleum substance combined with targeted in vitro tests may be used to predict the potential to cause prenatal developmental toxicity. We have tested the usefulness of a battery of in vitro alternative tests that combines the embryonic stem cell test (EST), the zebrafish embryotoxicity test (ZET), and the aryl hydrocarbon receptor (AhR) reporter gene assay to evaluate the developmental toxicity potency of petroleum substance extracts according to their PAH content ranging from 0 to 48%. The results in vitro show that some petroleum substance extracts' containing PAH may affect developmental toxicity. Potency is proportional to PAH content, especially of 3- to 7-ring PAHs. Further studies confirmed the role of AhR in mediating the developmental toxicity of petroleum substance extracts containing PAH. It was noted that adding an AhR antagonist counteracted the previously observed developmental effect.

The role of metabolism in the developmental toxicity of petroleum substance extracts containing PAH was also investigated (Kamelia et al. 2019a; Kamelia et al. 2019b; Kamelia et al. 2019c; Kamelia et al. 2020). This indicated that the developmental toxicity associated with petroleum substance extracts is caused by the presence of 3- to 7-ring PAHs, and the observed developmental effects are partially AhR-mediated.

These results are important because they further understanding of the role of PAHs in the prenatal developmental toxicity tests that REACH requires for petroleum products. It confirms that potency is associated with collective PAH types and levels, and that removal of PAHs during refinement of petroleum substances is crucial to eliminating this hazard.

3Rs Benefit

The results of these studies and further development of animal-free in vitro tests may reduce the need for regulations that require animal tests to be conducted in two species (Kamelia et al. 2019a; Kamelia et al. 2019b; Kamelia et al. 2019c; Kamelia et al. 2020).

Novel ecotoxicology methodologies

Eco21 Strategy

In 2018, Shell refreshed its strategy for the exploration of new approach methodologies in the field of ecotoxicology known as Eco21. This exercise continued in 2019 as prioritised areas were worked on. The Shell Eco21 strategy involved mapping the various applications of vertebrate use for ecotoxicology assessment. During this process, research gaps were identified that were of specific relevance to Shell. Research into non-vertebrate ecotoxicological assessment was prioritised. Through Shell leadership in an industry consortium, methods for dosing UVCBs and poorly soluble substances in high-throughput in vitro testing systems are being explored at Texas A&M University.

3Rs Benefit

This initiative addresses an obstacle to the uptake of non-vertebrate testing of UVCB petroleum products that make up about 25% of substances on national chemical registries.

EcoToxChip

The EcoToxChip programme is a multi-institutional and multi-sector collaborative research programme aimed at developing, testing, validating and commercialising EcoToxChips (based on quantitative polymerase chain reaction arrays). This will consist of more than 300 genes covering key toxicity pathways of regulatory concern in three key vertebrate model species, (fish, frog and bird), that are used globally in ecological risk assessment.



A data evaluation tool (EcoToxXplorer.ca) has been developed to allow end users to upload EcoToxChip data and interpret their results for the characterisation, prioritisation and management of environmental chemicals and complex mixtures of regulatory concern.

Shell has been collaborating on the project, advising the research team on end-user needs for the EcoToxChip. During 2019, Shell Health in Houston hosted a postdoctoral fellow from the University of Saskatchewan to test and validate an early version of the EcoToxChip.

3Rs Benefit

EcoToxChips will eventually be developed as replacements for three native species of fish, frog and bird in North America.

ADOPT AND ENABLE

We aim to adopt our research advances, understanding and external good practice into Shell's ways of working. Shell incorporates the advances and insights into its methods of assessing internal hazard and exposure.

Building models with existing information

3Rs elements – reduction

After successfully developing the use of computer models for several outcomes including skin irritation, Shell has used the experience to help develop a model for respiratory irritation.

The RespiraTox Challenge, funded by the UK's NC3Rs CRACK IT and partly sponsored by Shell, developed a quantitative structure-activity relationship (QSAR) computer simulation model to predict the potential of individual compounds to cause irritation in the respiratory tract. The model was further developed in 2019 (Wehr et al. 2019a; Wehr et al. 2019b).

QSAR models rely on high-quality datasets. Because there is no specific in vivo toxicity testing method for respiratory irritation, the model was developed using empirical information from databases and animal studies. Results from human volunteer studies for around 100 compounds were also included. The final project dataset included more than 2,500 respiratory irritant and 600 non-irritant compounds which were cross-referenced with physical-chemical and structural properties using machine learning algorithms. Structural

properties include the molecular structure, and physical-chemical properties include water-solubility. Although the applicability domain for the model has not been fully characterised, it is worth noting that the use of physical-chemical properties achieves better predictions than the use of structural information alone. The current approach will be further refined and improved (for example, by differentiating between sensory and tissue irritation).

3Rs Benefit

The final model will be provided within a user-friendly tool to promote its uptake by toxicologists, regulators and others, in order to reduce animal testing in acute, sub-acute, sub-chronic and chronic studies that assess respiratory irritation.

(<https://www.item.fraunhofer.de/en/press-and-media/news/respiratox.html>).

The work presented here was supported by the NC3Rs CRACK IT Challenge 28: RespiraTox and Shell.



Use of Interspecies Correlation Estimates

Shell has been investigating how interspecies correlation estimate (ICE) models could avoid the need to generate new data from vertebrates (fish) when addressing hazard assessment questions. ICE models describe mathematical relationships between pairs of species responses (e.g. LC50 values) that can be used to predict toxicity from surrogates to untested species. Previous work has indicated their potential application to dispersants (Bejarano 2019) whilst future efforts will focus on encouraging wider and greater regulatory acceptance of their application.

3Rs Benefit

This work increases the output from data already collected from animals, and may reduce the need for further animal testing.



Strategy to reduce fish testing

In 2019 Shell engaged in two key activities under its Fish Strategy to reduce the use of fish in routine whole effluent toxicity (WET) testing of effluent discharges in North America.

The first activity was a historical review of WET data from 20 different Shell facilities with a view to using the pooled data to help understand the relative sensitivity of fish over invertebrates. The data can also help demonstrate the safety of Shell's effluent discharges over time, and the potential performance range required of non-vertebrate and alternative tests.

Such data can also be used when renewing discharge permits to help justify a reduction in the frequency of testing thereby reducing vertebrate use.

The second activity was conducted through HESI's Animal Alternatives for Ecological Risk Assessment Committee, where Shell has led efforts to plan a technical workshop on WET effluent testing to be held in 2020. The workshop will seek to develop a roadmap for acceptance and implementation of alternative approaches for effluent testing in North America.

3Rs Benefit

This work may encourage wider adoption of non-vertebrate testing for effluent testing.

EXTRAPOLATE AND ELIMINATE

One way to minimise or eliminate animal testing is by using existing data and prediction models. Information from multiple sources can be integrated to improve insights, for example in areas such as risk assessment.

Modelling existing data for better risk assessment

In addition to QSAR models, Shell has also sponsored work involving benchmark dose modelling (BMD), a software tool developed by the US Environmental Protection Agency (EPA). This software uses several mathematical models to fit dose-response data and to define a point of departure (POD) in order to establish a safe dose of exposure (i.e. reference value (RfD or RfC), see glossary) for health effects.

This model was applied to the wide range of haematological effects of occupational benzene exposure (decreased blood cell count and acute myeloid leukaemia). Applying the BMD software to decreased blood cell count to establish RfDs would protect exposed individuals from both decreased blood cell counts and more serious manifestations of toxicity. Results of this investigation, combined with previous studies, indicate alignment with several models and highlight a role for Bayesian Models in deriving RfDs for chemicals with complex health effects (North et al. 2019).

3Rs Benefit

Pooling of data maximises the use of existing results from animal testing, providing a better understanding of hazard data, read-across, and better predictions. This reduces the need for further animal testing or at least shows where targeted testing is required.



DISSEMINATE AND DISCUSS

To further the replacement, reduction and refinement of animal use in assessing chemical safety, Shell publishes the results of research and development, presents data and ideas in professional fora and engages with regulators and academia. By doing so, Shell aims to instil a culture of care at the highest scientific and practical level among stakeholders. It also seeks to gather feedback and promote an exchange of ideas that will generate a wide acceptance of Shell's activities around the 3Rs.

Improved hazard assessment for toxicological endpoints

3Rs element - reduction

It is important to support ongoing research efforts. In 2019, Shell contributed to update the data base of health and environmental effects of two widely used industrial chemicals (Banton 2019; Petry 2019). Such updates aid the development of more accurate occupational exposure guidelines and toxicity reference values for the general public. Additionally, as old information is updated, new methodologies like QSAR information can be integrated with more traditional approaches, (in vitro/in vivo data), to produce a more comprehensive picture for risk and hazard assessment.

In order to improve hazard knowledge, unpublished animal data was re-evaluated by comparing it to previously published work. Studies of the health impacts induced by hydrocarbon solvents indicate that acute central nervous system (CNS) effects are the most sensitive health outcomes for these types of substances. These studies, based

on the most potent substance to produce CNS effects, provide empirical evidence to support current recommended occupational exposure values in the US and Europe (McKee et al. 2019), and discourage the generation of new animal data on related substances.

The update to DCPD (Petry et al. 2019) and the BMD modelling of occupational benzene exposure (North et al. 2019) were both presented at the annual 2019 EUROTOX meeting in Helsinki, Finland. We presented the results of the QSAR model for respiratory irritation prediction (Wehr et al. 2019a) at the Society of Toxicology annual meeting in Baltimore, Maryland and at the Annual Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology (Wehr et al 2019b).

3Rs Benefit

Disseminating existing and unpublished animal data maximises the use of existing findings from animals, allowing a better understanding of hazard data, read-across, and better predictions. This reduces the need for further animal testing or at least shows where targeted testing is needed.

Novel methods for assessing carcinogenicity in petroleum products

3Rs element - replace

Mineral oils' potential for carcinogenicity is related to their polycyclic aromatic compound (PAC) content. Their assessment must be targeted because aromatic compounds found in mineral oils are of two types: they either have 1-2 highly alkylated aromatic rings or 3-7 PAC rings. Discriminating between the two types is toxicologically

relevant because PACs with 1-2 highly alkylated rings are not carcinogenic whereas those with 3-7 ring PACs are. The DMSO-based IP346 method enables discrimination between these two types. Specifically, DMSO preferentially extracts PACs with more than three rings and excludes PACs with less than three rings and long side chains. The dissemination of the original research data supports the continuous use of an industry standard (IP346) which replaced an animal test that was previously considered the “gold standard” (Carrillo et al. 2019).

During the past year, Shell has supported the use of the IP346 method with additional studies that add mechanistic information to reinforce the predictive capabilities of animal-free alternative test methods. To this end, ToxTracker (a battery of in vitro tests), comprised of six validated mouse embryonic stem cell lines, was used to test 18 petroleum substances. ToxTracker uses reporter genes to convey information on DNA damage, oxidative stress, and protein damage within a single test. Chemical classifications of genotoxicity from ToxTracker agreed with results from animal-free alternative test methods such as the Modified Ames test, as well as in vivo genotoxicity results. This project further refined the ToxTracker protocol by optimising it to fit the complexities of petroleum UVCB substances around inherent autofluorescence and metabolic activity (Hendriks et al. 2019).

The results of the ToxTracker assay were presented in 2019 at the Society of Toxicology annual meeting in Baltimore, Maryland.

3Rs Benefit

The use of cell lines as an alternative to vertebrates for the assessment of carcinogenicity in humans.

EnviroTox database and ecological threshold of toxicological concern (Eco-TTC)

3Rs element - replace

In 2019, the dissemination of the EnviroTox database and associated web-based Eco-TTC tool continued. The Threshold of Toxicological Concern concept is a well-established risk assessment tool that allows a human exposure concentration with negligible risk to be determined in the absence of chemical-specific data. Similarly, the Eco-TTCs summarise the wealth of ecotoxicological information in the curated EnviroTox database by giving the predicted no-observed-effect concentrations (PNECs) of diverse chemical substances, expressed as statistical (probability) distributions. The Eco-TTC approach maximises resources by using existing ecotoxicity knowledge and extrapolating to data-poor chemicals. It supports read-across, which is helpful for low-production-volume chemicals with little data or in the early tiers of the risk assessment process. This in turn enables rapid decision making. The EnviroTox database and Eco-TTC tool received significant interest from the scientific and regulatory community, and efforts will continue to gain acceptance and uptake of the database and tool by developing publications with case studies that highlight their use, and through demonstrations and training at conferences. The work was presented at most of the regional Society of Environmental Toxicology and Chemistry (SETAC) meetings (Europe, North America and Latin America; see Otter et al 2019, Bejarano et al 2019a, Embry et al 2019, Bejarano et al 2019b).

3Rs Benefit

Better use of existing data may help reduce the need for further animal testing on similar chemicals.

General assessment approaches improvement

3Rs element - replace

Better, more targeted risk assessment and management practices can lead to an overall reduction in the need for new animal test data. Approaches that build regulatory confidence and address outstanding challenges can ensure reliability and faster uptake of appropriate alternatives.

During 2019, Shell has been active in identifying (Fairbrother et al 2019) and addressing areas that might benefit from such approaches. One key area for Shell has been the risk assessment of offshore discharges (Lyon et al 2019, Hughes et al 2019, Karmen and Smit 2019); other effluent streams (Cailleaud et al 2019); spills (Camu and Smit 2019); and groundwater contamination (Kilgour et al 2019, Ajaero et al 2019). In addition to developing guidance to assist with testing the toxicity and bioaccumulation of 'difficult' substances (Gouin et al 2019, Birch et al 2019), we keep an eye out for potential future developments that could eventually lead to new requirements (Oziolor et al 2020), and the communication of these approaches to the wider public (Menzie et al 2019).

Improved exposure assessment

3Rs element - reduce

Estimates of exposure estimates are important for prioritising hazard assessment by providing a clear idea of which chemicals we are more likely to be exposed to. This approach concentrates on those chemicals produced at high tonnages, potentially reducing the need

for animal tests by targeting those chemicals which have a wider presence in our environment.

In 2019, Shell's Health Exposure Science Team continued its engagement with the International Society for Exposure Science, European Chapter, through the following activities: co-authoring a strategy publication for the development and establishment of exposure science in Europe (Fantke et al. 2019); participating in the ISES-Europe workshop on July 4-5 at the RIVM offices in Bilthoven, the Netherlands¹; presenting at the global joint conference of the International Society for Exposure Science and the International Society for Indoor Air Quality in Kaunas, Lithuania²; and continuing participation in two ISES-Europe working groups: Education, Training and Communication³ Exposure Models⁴.

3Rs Benefit

By engaging in the above-mentioned activities Shell seeks to promote effective risk management through personalised and rapid exposure assessment, and to reduce future animal use through exposure-based waiving and/or adaptation of hazard testing programmes. The activities will also help the company to build capability for future exposure science needs.

1 <https://ises-europe.org/events/ises-europe-2019/workshop-outcome>

2 http://isesisiaq2019.org/wp-content/uploads/2019/08/ISESISIAQ2109-program-8.17.19_online2.pdf

3 <https://ises-europe.org/group/education-training-and-communication>

4 <https://ises-europe.org/group/exposure-models>

SHELL USE OF ANIMALS FOR TESTING IN 2019

In line with standard industry practices, Shell reports on the activities of Shell-owned and Shell-operated companies. Testing programmes that are supervised by industry consortia in which Shell or Shell joint ventures (JVs) participate are reported separately. Shell reports all experimental animal use on a 100%-basis (each animal is reported in Shell's figures, even if the testing programme is undertaken jointly with other companies through, for example, industry consortia).

Testing data is collected from internal sources and from reports provided by external testing laboratories.

Table 1 shows the total number of laboratory animals used in procedures from 2015-2019. For 2019, the total number of vertebrates (including mammalian, fish and amphibian species) was 37,354. This total is comparable to the number reported in 2017-18. In 2019, the use of fish for regulatory mandated effluent testing in North America remained the most significant contributor to the total number of animals used by Shell.

Table 1 Number of laboratory animals used worldwide, 2015 - 2019

Animals used	Test commissioned	Number of animals per year				
		2015	2016	2017	2018	2019
Fish	Shell	76,476	42,926	32,732	34,499	35,800
Fish	Industry consortia	2,720	2,285	0	1,600	0
Fish	Joint ventures	6,260	10,140	1,920	720	780
Amphibians	Shell	5,770	12,180	17	0	0
Rodents	Shell	72	0	0	105	215
Rodents	Industry consortia	9,908	767	1,787	765	547
Rodents	Joint ventures	0	0	0	0	0
Rabbits	Shell	3	0	0	0	6
Rabbits	Industry consortia	20	24	3	0	6
Rabbits	Joint ventures	0	0	0	0	0
TOTALS		101,229	68,322	36,459	37,689	37,354

Explanatory notes:

Industry consortia are groups of companies (including Shell) that co-operate, usually within the framework of an industry trade association, to share available data and the costs of testing programmes on particular chemicals or groups of chemicals.

Joint ventures include JVs where Shell has operational control. In instances where work was placed for a JV through an industry consortium, the data are reported under industry consortia.

In 2019, the majority of mammalian testing was conducted through industry consortia. The benefit of performing animal testing through consortia is that duplication of tests is avoided. Standardisation can also produce opportunities for read-across approaches that fill data gaps and suggest further ways to reduce animal testing within substance groupings.

Although Shell reports animal numbers on a 100% basis, the specific impact of working through consortia over Shell's total animal numbers is shown in Table 2.

If the number of animals used in a consortium study is divided by the total number of consortium partners, a relative "Shell share" of the total number of animals used is obtained. The calculation shows that from a total of 553 mammals used in consortia, the Shell share was around 55 mammals. This clearly demonstrates how working in consortia helps reduce animal numbers.

Table 2 Mammalian species used in consortia for testing

Species	Total number	Number used in consortia	"Shell share" of animals used in consortia *
Rats	692	507	51
Mice	40	40	4
Rabbits	12	6	<1
Guinea pig	30	0	-
TOTAL	774	553	55

* about 10 members per consortia

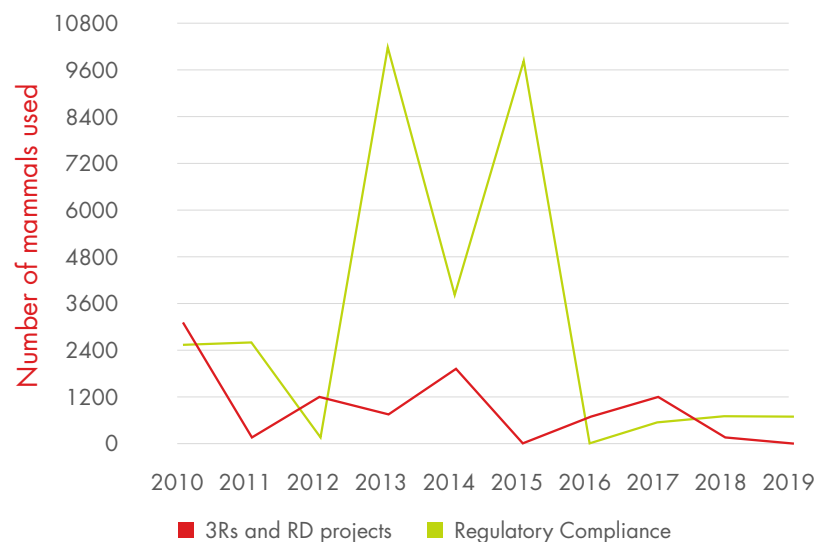
PURPOSE OF TESTING ON ANIMALS IN 2019

Shell indicates the purpose for animal testing using the categories "3Rs and research" and "regulatory compliance". The purpose of regulatory compliance is self-explanatory. The purpose of 3Rs and research is defined as data that is generated to understand the health and environmental hazards of a product and not collected for regulatory reasons, and/or is developed for research intended to advance the 3Rs. This may include the generation of detailed information on the mechanism of toxic action. This mechanism of action can inform the relevance of the used animal model for human risk assessment, or help the development of novel non-animal testing methods.

Shell applies smart and combinatorial testing strategies because it is using the 3Rs concepts to promote animal welfare. For example, when obliged to conduct an animal test for regulatory compliance, there might be an opportunity to combine the mandated test with a research project which would maximise the use of information obtained from the animals used. This research project would typically generate data to advance 3Rs methodologies or enhance the information on Shell's chemical portfolio.

As shown in Figure 1, since 2010 the number of mammals used for projects on 3Rs and research has remained stable. However, the number of animals used for regulatory compliance fluctuates from year to year. This is because of changing regulatory demands, which can be impacted by global regulations coming into force. In 2019, a significant amount of animal-intensive tests began because of REACH. This will result in a high figure for 2020 because these tests will be completed in 2020. This peak will be in the thousands of animals.

Figure 1 Purpose of testing in mammalian species



From 2010 to 2019, 58% of all mammals used in regulatory compliance tests worldwide were used solely to comply with REACH. This figure is likely to increase as more animal-intensive tests are required by the European Chemicals Agency (ECHA).

As seen in Figure 2, since 2010, 44% of all tests on mammals for regulatory compliance, 3Rs and research and development have been for REACH purposes. The impact of REACH on the total number of mammalian species is significant (44%), when compared to non-EU regulatory frameworks or 3Rs and research and development figures. Since 2010, 23% of animals used by Shell have been tested for 3Rs and research and development (RD) projects.

Figure 2 Impact of REACH on mammalian species (2010-2019)

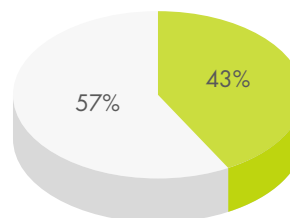
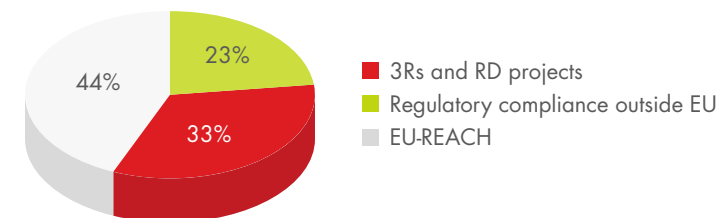


Figure 3 Purpose of testing on mammalian species (2010-2019)



Shell sometimes tests to advance 3Rs methods or for research and development to understand the health and environmental hazards of a product when testing is not mandated for regulatory compliance. Research and development data are also used or generated to advance 3Rs methods. The data obtained may include detailed information on the mechanism of toxic action that can inform assessments of the relevance of animal models that are currently used for human risk assessment. This type of data can be used to group chemicals into “categories” to reduce the mandated tests that would otherwise be required for each individual member of the category.



TESTING IN FISH SPECIES

In 2019, no fish were used worldwide for 3Rs development activities. A small number, 450 fish, were used for a project to develop a fish feed product. Overall fish numbers remained stable with the 2018 data. Most of the non-mammal-vertebrate testing in 2019 stemmed

from fish testing that was directly required under regulations relating to whole effluent toxicity assessment (see Table 3). The primary driver for Shell's fish use, accounting for over 99% of all fish used in 2019, remains whole effluent toxicity testing requirements for discharge permits in North America and hazardous waste disposal in California.

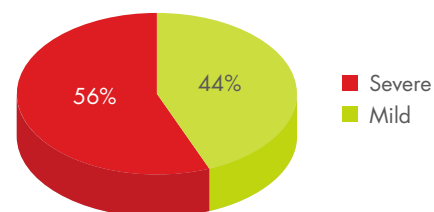
Table 3 Use of fish, 2015 - 2019

Purpose of Test	2015	2016	2017	2018	2019
3Rs and Research ¹	18,589	8,480	274	0	450
Regulatory Compliance	66,867	46,871	34,378	36,819	36,130
TOTAL	85,456	55,351	34,652	36,819	36,580

* 3Rs and research: data is required to understand the health and environmental hazards of a product and is not collected for direct regulatory purposes. This testing is also performed to help Shell understand the potential implications of anticipated future regulatory requirements or applications for new permits (discharges).

Fish studies were relatively evenly split between acute mortality effluent tests that would be rated 'severe', and chronic studies that would be rated 'mild' under Directive 2010/63/EU (see Figure 4).

Figure 4 2019 Fish Testing by Severity



CONCLUSIONS

Regulatory requirements are the main driving force for conducting tests on mammals (EU REACH) and fish (North America). The use of fish for regulatory mandated effluent testing in North America remains the most significant contributor to the total number of animals used by Shell. For this reason, Shell will continue to focus on promoting 3Rs methodologies that will eventually be accepted by regulators.

Over the past 10 years, EU-REACH has been the main regulatory framework demanding tests on vertebrate species. Although the numbers have been relatively stable since 2016, we expect a significant increase in numbers because of ECHA's strict interpretation of the law on mandated tests and its rejection of industries' read-across proposals. Shell will always comply with the law and will at the same time try to reduce its use of animals for regulatory compliance by leveraging the best science available.

ABOUT THE PANEL

In 2001, Shell formalised its practices on animal testing by creating a more structured management process and by better communicating its position internally and externally. An external Animal Welfare Panel was established to provide independent scrutiny of, and support for, Shell's activities in this area.

TERMS OF REFERENCE OF THE PANEL

Shell invites individual Panel members to serve on the Panel for a period of three years, with the possibility of being invited to serve for a second term of three more years. The Panel recommends candidates who could be invited by Shell to join the Panel, either as replacements for current members when their term has been completed, or to supplement the current Panel membership.

The Panel meets twice a year with key Shell personnel. It does not verify the accuracy of the data underlying the Report. Besides assessing Shell's reporting on animal testing, the Panel offers observations and advice on the company's performance with respect to the 3Rs. In recognition of their time and expertise, Panel members receive an honorarium and reimbursement of travel and accommodation expenses.



PANEL MEMBERSHIP IN 2019

Jim Bridges (Emeritus Professor of Toxicology and Environmental Health at the University of Surrey, UK)

Jim Bridges held previous positions in the University of Surrey, including Dean of Science and founding head of two large health research and teaching institutes. He has published around 400 papers and reviewed and trained 98 PhD students. He is a founder of both the British Toxicology Society and EUROTOX. His work for the EU included serving as chair of two scientific committees – Emerging and Newly Identified Health Risks; and Toxicity, Ecotoxicity and the Environment – and being a member of several working groups on future risk assessment methodology that have addressed alternatives to animal testing.

Catherine Willett (Director, Science Policy, the Humane Society of the United States)

Kate Willett began her career at the Massachusetts Institute of Technology as a developmental biologist studying embryology using the zebrafish as a model system. She then joined a start-up company that pioneered the use of zebrafish for preclinical drug testing. Since 2006, she has focused on the science, policy and regulatory aspects of replacing animals as the basis of chemical safety assessment, first as Science Policy Advisor for People for the Ethical Treatment of Animals (PETA), and more recently at the Humane Society of the United States as coordinator of the Human Toxicology Project Consortium (HumanToxicologyProject.org). She has published a number of papers on non-animal approaches and advises international companies and governments on the regulatory use of non-animal methods.

Robert Hubrecht OBE (Until January 2020, Chief Executive and Scientific Director of the Universities Federation for Animal Welfare and the Humane Slaughter Association)

Robert Hubrecht is an ethologist with an interest in animal welfare. Prior to joining the Universities Federation for Animal Welfare, he held positions at the Open University and Cambridge University in the UK. His research has included studies of the behaviour, physiology and natural history of farm animals, New World primates (in captivity and the wild), and the welfare of kennelled dogs. He has served on numerous advisory committees, including the UK Animal Procedures Committee, the US National Research Council Distress Committee, and expert groups that advised on the development of UK and European legislation. He co-edited the 8th edition of The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals. In 2014, he authored the book: The Welfare of Animals Used in Research: Practice and Ethics.

Sarah Wolfensohn

Sarah is a veterinary surgeon, a Royal College of Veterinary Surgeons-recognised specialist in laboratory animal science and a European specialist in animal welfare, ethics and law. While in general practice, she became Named Veterinary Surgeon for a number of small pharmaceutical and biotech companies, before being appointed Head of Veterinary Services at the University of Oxford. She is now Professor of Animal Welfare at the University of Surrey's veterinary school and also runs an independent consultancy on animal health and welfare. She has published textbooks and numerous papers in the area of animal science and welfare, and is a member of the UK government's Animal Welfare Committee. She was previously a member of the UK Animals in Science Committee and the Animal Procedures Committee, and was closely involved in the UK government's development of its Animal Health and Welfare Strategy. She has served on several international committees and working groups that seek to refine animal use and improve welfare.

REFERENCES

- Ajaero C, Peru KM, Simair M, Friesen V, **Hughes S**, Chen H, McKenna A, Corilo Y, McMartin D, Headley JV (2019). Atmospheric pressure photoionization Fourier transform ion cyclotron resonance mass spectrometry characterization of oil sand process-affected water in constructed wetland treatment. *Energy & Fuels*, 33: 4420-4431.
- Banton MI, Bus JS, Collins JJ, Delzell S, Gelbke HP, Kester JE, Moore MM, Waites R, **Sarang SS**. (2019). Evaluation of Potential Health Effects Associated with Occupational and Environmental Exposure to Styrene - An Update. *J Toxicol Environ Health B Crit Rev*, 22 (1-4), 1-130
- Bejarano AC** (2019). Further Development and Refinement of Interspecies Correlation Estimation Models for Current-Use Dispersants. *Environmental Toxicology and Chemistry* 38(8): 1682-1691.
- Bejarano AC**, Barron MG, Connors K, Embry M, Fay K (2019a). Quantifying Conservatism in Ecological Threshold for Toxicological Concern and Chemical Toxicity Distributions: Case Study of Chemicals with Regulatory Water Quality Values. Presented at SETAC Toronto – SETAC North America 40th Annual Meeting, 3–7 November 2019, Toronto, Canada. Platform presentation.
- Bejarano, AC**, Embry MR, Barron M, Beasley A, Belanger SE, Bonnell M5, Brill J, Chang D, Connors K, De Zwart D, Halder M, **Hughes S**, Kienzler A, Norberg-King T, Otter R, Sanderson H, Wilson P (2019b). EnviroTox: a New Web-Based Application for Environmental Risk Assessment that Uses Ecological Threshold for Toxicological Concern. Presented at SETAC- Latin America; September 15-18, Cartagena Colombia. Poster presentation.
- Birch H, Redman A, Letinski DJ, **Lyon D**, Mayer P (2019). Determining the water solubility of difficult-to-test substances: A tutorial review. *Analytica Chimica Acta*, 1086: 16-28.
- Cailleaud K, Basseres A, Gelber C, Postma JF, ter Schure ATM, Leonards PEG, Redman AD, **Whale GF**, Spence MJ, Hjort M. (2019). Investigating predictive tools for refinery effluent hazard assessment using stream mesocosms. *Environmental Toxicology and Chemistry*, 38: 650-659.
- Camus L, **Smit MGD** (2019). Environmental effects of Arctic oil spills and spill response technologies, introduction to a 5 year joint industry effort. *Marine Environmental Research*, 144: 250-254.
- Carrillo JC, van der Wiel A, Danneels D, Kral O, **Boogaard PJ**. (2019) The selective determination of potentially carcinogenic polycyclic aromatic compounds in lubricant base oils by the DMSO extraction method IP346 and its correlation to mouse skin painting carcinogenicity assays. *Regulatory Toxicology and Pharmacology* 106:316-333.
- Embry MR, Barron M, Beasley A, Belanger SE, Bonnell MA, Brill J, Chang D, Connors K, De Zwart D, Halder M, **Hughes S**, Kienzler A, Norberg-King T, Otter R, Sanderson H, Wilson P (2019). EnviroTox Database: Overview and Applications. Presented at SETAC - Europe; May 26-30, Helsinki, Finland, Platform presentation.
- Fairbrother A, Muir D, Solomon KR, Ankley GT, Rudd MA, Boxall ABA, Apell JN, Armbrust KL, Blalock BJ, Bowman SR, Campbell LM, Cobb GP, Connors KA, Dreier DA, Evans MS, Henry CJ, Hoke RA, Houde M, Klaine SJ, Klaper RD, Kullik SA, Lanno RP, **Meyer C**, Ottinger MA, Oziolor E, Petersen EJ, Poynton EC, Rice PJ, Rodriguez-

Fuentes G, Samel A, Shaw JR, Steevens JA, Verslycke TA, Vidal-Dorsch DE, Weir SM, Wilson P, Brooks BW (2019). Toward sustainable environmental quality: Priority research questions for North America. *Environmental Toxicology and Chemistry*, 38: 1606-1624.

Fantke P, von Goetz N, Schlüter U, Bessems J, Connolly A, Dudzina T, Ahrens A, Bridges J, Coggins MA, Conrad A, Hänninen O, Heinemeyer G, Kephelopoulos S, McLachlan M, **Meijster T**, Poulsen V, Rother D, Vermeire T, Viegas S, Vlaanderen J, Zare Jeddi M & Bruinen de Bruin Y. (2019). Building a European exposure science strategy. *Journal of Exposure Science & Environmental Epidemiology*, 1-8

Gouin T, Salinas E, Coady K, Franco A, Hoke R, Hopp P, Hughes C, Leonard M, **Lyon D**, Sergent JA. (2019). TR 132 – An evaluation of the challenges and limitations associated with aquatic toxicity and bioaccumulation studies for sparingly soluble and manufactured particulate substances. ECETOC. <http://www.ecetoc.org/wp-content/uploads/2018/12/ECETOC-TF-132-Aq-tox-particulates.pdf>

Hendriks G, Derr RS, Racz PI, **Boogaard PJ**, Ketelslegers H. (2019). Adaptation of the ToxTracker reporter assay for the genetic toxicology assessment of petroleum products. Presented at: *Annual meeting of the Society of Toxicology, Baltimore, MD, 10-14 March 2019*. *Toxicologist* 58:137. Poster presentation.

Hughes SA, Naile J, Pinza M, Ray C, Hester B, Baum J, Gardiner W, Kallestad W, **Brzuzy L** (2019). Characterization of miscellaneous effluent discharges from a mobile offshore drilling unit to the marine environment. *Environmental Toxicology and Chemistry*, 38: 2811-2823.

Kamelia L, Brugman S, de Haan L, Ketelslegers HB, Rietjens IMCM, **Boogaard PJ**. (2019a). Prenatal developmental toxicity testing of petroleum substances using the zebrafish embryotoxicity test. *ALTEX*, 36:245-260.

Kamelia L, De Haan L, Ketelslegers HB, Rietjens IMCM, **Boogaard PJ**. (2019b) In vitro prenatal developmental toxicity induced by some petroleum substances is mediated by their 3- to 7-ring PAH constituent via activation of the aryl hydrocarbon (Ah) receptor. *Toxicol Lett*, 315:64-76.

Kamelia L, de Haan L, Ketelslegers HB, Rietjens IMCM, **Boogaard PJ**. (2019c). A battery of animal-free in vitro assays for evaluating prenatal developmental toxicity potency of highly complex petroleum substances. Presented at *EUROTOX 55th Congress of the European Societies of Toxicology, Helsinki, Finland 8-11 September 2019*. *Toxicol Lett*, 314:S166.

Kamelia L, De Haan L, Spenkelink B, Bruyneel B, Ketelslegers HB, **Boogaard PJ**, Rietjens IMCM. (2020). The role of metabolism in the developmental toxicity of polycyclic aromatic hydrocarbon-containing extracts of petroleum substances. *J Appl Toxicology*, (in press; online available).

Karman C, **Smit MGD** (2019). Whole effluent toxicity data and discharge volumes to assess the likelihood that environmental risks of offshore produced water discharges are adequately controlled. *Integrated Environmental Assessment and Management*, 15(4): 584-595.

Kilgour B, Mahaffey A, Brown C, **Hughes S**, Hatry C, Hamilton L (2019). Variations in toxicity and ecological risks associated with some oil sands groundwaters. *Science of the Total Environment*, 359: 1224-1233.

Lyon, DY, M Smit, B Lee, B Conley (2019). OTC-29835-MS: Modeled Environmental Risk of Offshore Drill Cuttings Discharges with Different Drilling Base Fluids. Offshore Technology Conference. doi:10.4043/29835-MS.

McKee RH, Nessel CS, **Carrillo JC**. (2019). An investigation of the acute central nervous system effects of n-decane. *Regulatory Toxicology and Pharmacology*, 107:104421.

Menzie C, **Hughes S**, Assis H (2019). Insight: Weight of evidence best way to manage chemical risks. *Bloomberg Environment & Energy*, <https://news.bloombergenvironment.com/environment-and-energy/insight-weight-of-evidence-best-way-to-manage-chemical-risks>

North CM, **Rooseboom M**, Dalzell A. (2019). Benchmark dose modeling for hematologic effects of occupational benzene exposure. Presented at EUROTOX 55th Congress of the European Societies of Toxicology, Helsinki, Finland 8-11 September 2019. *Toxicol. Lett.*, 314, S1-S309. Poster Presentation

Otter RR, Embry MR, Barron MG, Beasley A, Belanger SE, Bonnell MA, Brill J, Chang DT, Connors KA, De Zwart D, Fay KA, **Hughes SA**, Kienzler A, Norberg-King T, Sanderson H, Vallotton N, Wilson P (2019). EnviroTox Database: Overview and Applications. Presented at SETAC Toronto - SETAC North America 40th Annual Meeting, 3-7 November 2019, Toronto, Canada, Special Forum Publicly Accessible Data Analytic Tools for Environmental Toxicology, Chemistry, and Risk Assessment. Platform presentation.

Oziolor E, De Schamphelaere K, **Lyon D**, Nacci D, Poynton H (2020). Evolutionary Toxicology - an informational tool for chemical regulation? *Environmental Toxicology and Chemistry*, 39(2): 257-268.

Petry T, Aygun Kocabas N, Mahni B, Rushton E, **Rooseboom M**, Synhaeve N, Martin G. (2019). A review of the toxicological information available for dicyclopentadiene (DCPD) pertinent to its assessment to potentially cause endocrine disruption. Presented at EUROTOX 55th Congress of the European Societies of Toxicology, Helsinki, Finland 8-11 September 2019. *Toxicol. Lett.*, 314, S1-S309. Poster Presentation.

Rooseboom M. Risk assessment in the petrochemical industry. Lecture given at IRAS Utrecht University, The Netherlands. 12 November 2019.

Wehr M, Karwath A, **Sarang SS**, **Rooseboom M**, **Boogaard PJ**, Escher SE. (2019a). Development of a QSAR model to predict respiratory irritation by individual constituents. Presented at: *Annual meeting of the Society of Toxicology, Baltimore, MD, 10-14 March 2019*. *Toxicologist* 58:200. Poster Presentation.

Wehr M, Karwath A, **Sarang SS**, **Rooseboom M**, **Boogaard PJ**, Escher SE. (2019b). Development of a QSAR model for respiratory irritation. Presented at *85th Annual Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology (DGPT)*, Stuttgart, Germany, 25-28 February 2019. *Naunyn-Schmiedebergs Arch Pharmacol*, 392(S1):67. Poster Presentation.